Rheologic changes of hypothermic preserved red blood cells

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Chapter 6

The effects of cryopreservation on red blood cell rheologic properties

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Abstract

In transfusion medicine, cryopreserved RBCs are an alternative for refrigerated stored RBCs. Less is known about the rheologic properties of cryopreserved RBCs. In this study the aggregability and deformability of HGM cryopreserved RBCs that were post-thaw stored in SAGM solution were compared to that of refrigerated stored and fresh RBCs. Fresh RBCs were obtained from healthy volunteers while leukoreduced refrigerated stored and cryopreserved RBC units were obtained from the Sanquin Blood Bank. RBCs were tested for aggregability, deformability and various hematologic variables. The AI of cryopreserved RBCs was considerably reduced, compared to fresh and refrigerated stored RBCs. The EI of stored RBCs was significantly enhanced over a shear stress range of 2.0 to 50 Pa compared to fresh RBCs. No significant differences in EI between cryopreserved and 21- or 35-day refrigerated stored RBCs were observed. The osmotic fragility, hemolysis, MCV and MCHC of cryopreserved RBCs were markedly altered, compared to fresh and refrigerated stored RBCs ($p < 0.05$). The ATP content of cryopreserved RBCs was similar to fresh and 3-or 21-day refrigerated stored RBCs. These findings suggest that although cryopreserved RBCs are more fragile than fresh and refrigerated stored RBCs, the freeze-thaw-wash process did not did not adversely affect the aggregability, deformability or the ATP content of cryopreserved RBCs. Based on these rheologic properties, we conclude that cryopreserved RBCs are a valuable alternative to refrigerated stored RBCs.

6.1. Introduction

In transfusion medicine RBCs are refrigerated stored for a maximum of 5 to 6 weeks before being discarded. Alternatively, cryopreservation enables storage of RBCs for years.[1,2] Cryopreservation is currently a valuable approach for long-term storage of RBCs from donors with rare blood groups and for military deployment.[3-5] However, stockpiling cryopreserved RBCs can also be beneficial in emergency or clinical situations, where the demand exceeds the supply of RBCs. At the moment, the shelf life of HGM cryopreserved RBCs has been approved for up to ten years.[3,4]

Usage of glycerol as a cryoprotectant requires a deglycerolization washing procedure post-thaw to prevent hemolytic transfusion reactions and renal failure after infusion.[6-8] Usage of the ACP 215 device (Haemonetics, Braintree, MA) to glycerolize and deglycerolize RBC units has minimized the risk of bacterial contamination. As a result, the post-thaw storage time of RBCs has extended to 2 days in SAGM solution and to 14 days in AS-3 respectively.[9] Current regulations require that cryopreserved RBCs have a post-thaw recovery of at least 80% and that the hemolysis in the RBC unit remains below allowable levels (i.e., 0.8% in Europe and 1% in the United States). Additionally, at least 75% of the cryopreserved RBCs should remain within the circulation 24 hour after infusion.[3,4]

Current regulations however, do not specifically address the quality of stored RBCs.[10] The quality of HGM cryopreserved RBCs is primarily dependent on the pre-freeze and post-thaw storage time as well as on the anticoagulant and additive solution used. The duration of frozen storage per se, however, minimally attributes to cellular damage.[1,2,11] Previously it was shown that cryopreserved RBCs have no significant loss of cellular ATP content or CD47 antigen expression on the external RBC membrane during 24 hours of post-thaw storage in AS-3. Moreover, when the pre-freezing storage time was limited to 3 days, no 2,3-DPG loss, microvesiculation or PS externalization was observed after 24 hours of post-thaw storage.[1,12] Cryopreserved RBC units usually contain low potassium and free Hb content due to the removal of these extra cellular substances during washing. [9]

The aggregability and deformability of cryopreserved RBCs remain to be elucidated. These rheologic properties are important determinants of the blood flow and hence the oxygenation of the tissues.[13] In the venous system RBCs are able to form linear aggregates (so called Rouleaux) or more complex multi-cellular aggregates at low shear rates.[14] Normally, the blood flow is sufficient to disperse these aggregates. However, under

pathologic conditions stronger and larger aggregates may form that are more resistant to dispersion by the blood flow.[15] In the microcirculation, the RBC ability to deform due to applied forces, make these cells capable of passing narrow capillaries. A high deformability of RBCs and a rapid recovery of the normal shape are essential factors for maintaining tissue perfusion and cell survival.[16,17] Transfusion of rheologic impaired RBCs may hinder or obstruct the blood flow in the microcirculation, leading to reduced tissue perfusion, ischemia or infarction.[13,18-20]

In the Netherlands, leukoreduced RBCs are refrigerated stored in SAGM preservation solution for a maximum of 5 weeks. In chapter 4 we demonstrated that refrigerated storage minimally affected the RBC ability to aggregate and deform, even after 5 weeks of routine blood bank storage. Cryopreservation offers the advantage of storing RBCs for prolonged periods of time. However, once thawed the shelf life of RBCs is limited. In the current study, the aggregability, deformability and other hematologic variables of HGM cryopreserved RBCs that were post-thaw stored in SAGM solution for 2 days were assessed and compared to those of refrigerated stored and fresh RBCs.

6.2. Materials and Methods

RBC preparation and processing

Human blood (50 ml ± 10 %) was collected after informed consent from ten healthy volunteers and anticoagulated with 7 ml of CPD. Functional measurements were performed with whole blood, whereas the rheologic features were determined with washed RBCs. Briefly, whole blood was washed by centrifugation at 1100 x *g* for 12 minutes and decanted three times with PBS pH 7.4. The final Hct of the RBC solution was set between 45% and 60 % and all measurements were performed within 3 hours after donation.

Ten leukoreduced RBC units stored in SAGM solution were obtained from the Sanquin Blood Bank and refrigerated stored according to standard blood bank procedures as described in chapter 4. These RBC units, which were released for use on day 3 after donation, had a Hct of 45% to 60 % and contained fewer than 10^6 leukocytes per unit. In general, the average storage time of RBC units that are used in transfusion medicine is approximately 21 days.[21,22] For analysis samples were aseptically withdrawn from the RBC units at 3, 21, and 35 days of refrigerated storage, reflecting short-, average- and long term stored RBCs, respectively.

Ten cryopreserved RBC units were obtained from the Sanquin blood bank and stored in polypropylene tubes at 2 to 6° for 48 hours. These RBC have been cryopreserved according to the HGM freezing method and post-thaw resuspended in SAGM solution as described previously.[9] Briefly, leukoreduced refrigerated stored RBC units were centrifuged at 3200 x *g* for 5 minutes to remove the SAGM solution. Subsequently, the RBCs were glycerolized to a final concentration of 40% glycerol via the Haemonetics ACP-215 device.[23] All glycerolized RBC units were frozen and stored at –80 ± 10°C in a mechanical freezer for 34.1 ± 20.5 months. Cryopreserved RBC units were thawed in a temperature-controlled water-bath of 40°C, until the units reached a temperature between 25°C and 30°C. Subsequently, thawed RBCs were deglycerolized via the Haemonetics ACP-215 device and resuspended in SAGM solution. The supernatant osmolarity of all cryopreserved units was below 400 mOsm/ kg H_2O, indicating an efficient removal of glycerol.

Rheologic features

RBC aggregability and deformability were monitored in vitro by the LORCA (R&R Mechatronics, Zwaag the Netherlands).[24,25] Aggregation was induced by the addition of 10% HES (MW 200-kDa). Briefly, RBCs suspensions were centrifuged for 1 minute at 3500 x *g* and the supernatant was discarded. RBCs were resuspended in 10% HES 200-kDa solution (Fresenius, Bad Homburg, Germany) to obtain a Hct of 32 ± 6%. Aggregability was tested with 1 ml of the RBC suspension. RBC aggregation was monitored after complete disaggregation under increased shear stress. Both the aggregation measuring procedure and the subsequent analyses were computer controlled. Aggregability of RBCs was expressed by the AI, where a larger AI reflects an increased ability to aggregate. The AI was determined after correcting the Hct in all the samples to a constant value of 45%. The kinetics of aggregation ($T_{½}$) was expressed by the time necessary to induce 50% aggregation.

The deformability of RBCs was determined with RBC suspension that were diluted 1:100 in PBS (pH 6.5), containing 5% PVP (MW 360 kDa, Sigma-Aldrich, Germany) and with a viscosity of 30 mPa.sec. One ml of the latter RBC suspension was inserted into the LORCA and the RBC diffraction pattern was recorded at various shear stresses at 36.8 ± 0.2 °C. The deformability of the RBCs, which is expressed by the EI, was determined by the LORCA from the size of the vertical (L) and horizontal (W) axes of the diffraction pattern according

to the formula: EI = (L-W) / (L+W). An increased EI at a given shear stress indicates greater RBC deformability. A deformability curve was obtained by plotting the calculated values for EI versus the corresponding shear stress. The deformability at two shear stress values were examined more closely; the deformability at a shear stress of 3.9 Pa, which reflects the rigidity of the cell membrane, and the maximal deformability at shear stress of 50 Pa.

Osmotic fragility

The osmotic fragility of RBCs, which reflects the membrane's ability to maintain structural integrity, was determined by diluting RBCs in PBS solutions ranging from 0.90% to 0.35%. RBCs with a Hct level of 30 to 35% were diluted 1:100 in each PBS solution, mixed and incubated for 30 minutes at 4°C, followed by centrifugation for 12 minutes at 1100 x *g*. The free Hb in the supernatant was measured by a spectrophotometer (PowerWave 200 spectrophotometer, Bio-Tek Instruments, USA). The concentration of PBS necessary to induce 50% hemolysis defined the osmotic fragility index of the RBCs.[26] With this method, a larger osmotic fragility index corresponds to more fragile cells.

Hematologic variables

The RBC MCV, the Hb concentration and the Hct were determined with a hematologic analyzer (Medonic CA 530-Oden, Sweden). The cytoplasmic viscosity of RBCs, which is determined by the MCHC,[16,27] was calculated by dividing the Hb concentration by the Hct.
The amount of free Hb in the RBC suspension was determined according to the Harboe method.[28] In short, cell supernatant was obtained by centrifugation of RBC suspensions for 1 minute at 3500 x *g*. The supernatant was diluted 1:10 in 0.01% sodium carbonate in a flat-bottom 96-well microtiter plate and mixed for 30 minutes. The free Hb concentration in the supernatant was determined by measuring the OD at 415 nm and correcting for the OD at 380 and 450 nm (PowerWave 200 spectrophotometer, Bio-Tek Instruments, USA), according to the formula OD = 2* (OD 415 nm) – (OD 380 nm) – (OD 450 nm). The hemolysis was expressed as a percentage of the total amount of Hb present in the RBC lysates.
To determine the RBC ATP content, RBC samples were incubated with 8% ice-cold trichloroacetic acid, in a ratio of 1:3, during a 30-minute period. The samples were

centrifuged for 1 minute at 3500 x *g* and the protein-free supernatant was neutralized with 1.5 mol/L sodium carbonate. Aliquots were stored at –80°C for later batch analyses of ATP. The ATP content was determined with a commercially available enzyme assay (Roche Diagnostics, Germany). For detection of ATP, light emission was measured at 560 nm by an illuminometer (Wallac 1420 Multilabel Counter, Perkin Elmer Life Sciences, Finland). Due to the detrimental influence of pre-freezing storage time on the RBC energy content,[1,11] ATP was determined in RBC units with a pre-freezing storage time that did not exceeded 8 days.

Statistical analysis

Statistical analysis was performed using statistical software (SPSS, version 16.0, SPSS Inc., Chicago, IL). Data were tested for normality with the Kolmgorov-Smirnov goodness-of-fit test. In the case of normally distributed data, differences between storage groups were demonstrated by using unpaired t-tests. Within storage groups, paired t-tests were performed to show differences over time. For none normally distributed data, the Mann-Whitney test was used to quantify differences between groups whereas the Wilcoxon signed ranks test was used to quantify differences within storage groups. Differences are considered to be significant with a two-tailed p value of less than 0.05. Results are presented as means ± SD.

6.3. Results

Rheologic features

The RBC ability to aggregate, as represented by the AI, was significantly reduced in cryopreserved RBCs when compared to both fresh and refrigerated stored RBCs (Table 6.1). During storage, the AI of refrigerated stored RBCs was significantly reduced, whereas the AI of cryopreserved RBCs remained stable. The $T_{½}$ of cryopreserved RBCs was significantly higher on Day 0, compared to refrigerated stored RBCs (Table 6.1). The latter observation indicated that aggregation formation immediately after deglycerolization was slightly slower. During storage, the $T_{½}$ of refrigerated stored RBCs was significantly reduced in accordance with the AI, whereas the $T_{½}$ of cryopreserved RBCs did not significantly change. It should be noted that the SD of both AI and $T_{½}$ obtained with

cryopreserved RBCs, were much larger than those of fresh and refrigerated stored RBCs. Probably the variability in (pre) freezing time may provide an explanation.

The RBC deformation curve showed a typical sigmoid shape over a shear stress range of 0.6- 50 Pa for all the tested RBC groups (Figure 6.1). Yet, the deformation curves of cryopreserved and refrigerated stored RBCs were significantly elevated over the shear stress range of 2.0 Pa to 50 Pa compared to fresh RBCs. The deformability of RBCs at a shear stress of 3.9 Pa and 50 Pa was not significantly different between cryopreserved and 21- or 35-day refrigerated stored RBCs (Figure 6.2). During post-thaw storage, the RBC deformability at a shear stress of 3.9 Pa and 50 Pa did not yield significant changes. However, during refrigerated storage the RBC deformability at a shear stress of 3.9 Pa slightly increased (from 0.35 ± 0.01 to 0.38 ± 0.01 EI; $p < 0.01$), whereas the deformability at a shear stress of 50 Pa slightly decreased (from 0.58 ± 0.01 to 0.56 ± to 0.02; $p < 0.01$).

Table 6.1. Aggregation behavior of fresh, refrigerated stored and cryopreserved RBCs.

Measure	Fresh	Refrigerated storage			Post-thaw storage	
		Day 3	Day 21	Day 35	Day 0	Day 2
AI (%)	45.3 ± 2.6	46.9 ± 2.4	46.0 ± 3.8	44.4 ± 4.5 †	36.0 ± 8.6 *† ‡§	36.9 ± 8.0 *† ‡§
T ½ (sec)	1.3 ± 0.2	1.03 ± 0.3 *	0.86 ± 0.21 *†	0.91 ± 0.12 *†	2.4 ± 1.7 †‡§	2.3 ± 2.4

Aggregation behavior was expressed as mean ± SD of ten units. Significant differences with a P- value < 0.05 were reported

* significantly different from fresh RBCs

† significantly different from day 3 refrigerated stored RBCs

‡ significantly different from day 21 refrigerated stored RBCs

§ significantly different from day 35 refrigerated stored RBCs

Table 6.2. Hematologic variables of fresh, refrigerated stored and cryopreserved RBCs

Measure	Fresh	Refrigerated storage			Post-thaw storage	
	Day 0	Day 3	Day 21	Day 35	Day 0	Day 2
MCV (fl)	89.1 ± 2.4	89.0 ± 2.4	91.1 ± 2.6 †	92.0 ± 2.8 *†‡	104.2 ± 10.2 *†‡§	106.4 ± 8.3 *†‡§
MCHC (mmol/L)	20.4 ± 0.4	20.7 ± 0.6	20.5 ± 0.6	20.2 ± 0.3	16.1 ± 0.6 *†‡§	16.0 ± 0.8 *†‡§
Osmotic fragility (%)	0.45 ± 0.03	0.48 ± 0.02	0.46 ± 0.02	0.47 ± 0.02	0.66 ± 0.05 *†‡§	0.67 ± 0.08 *†‡§
Hemolysis (%)	0.26 ± 0.04	0.24 ± 0.07	0.36 ± 0.12 *†	0.53 ± 0.24 *†‡	0.57 ± 0.26 *†‡	0.99 ± 0.28 *†‡§
ATP (μmol/gHb)	6.1 ± 3.1	4.6 ± 1.3	3.8 ± 1.1	2.1 ± 0.4 *†‡	4.5 ± 2.2 §	4.0 ± 1.9 §

Values were expressed as mean ± SD of ten units. ATP values were determined from seven RBC units. Significant differences with a P-value less than 0.05 were reported

* significantly different from fresh RBCs
† significantly different from day 3 refrigerated stored RBCs
‡ significantly different from day 21 refrigerated stored RBCs
§ significantly different from day 35 refrigerated stored RBCs

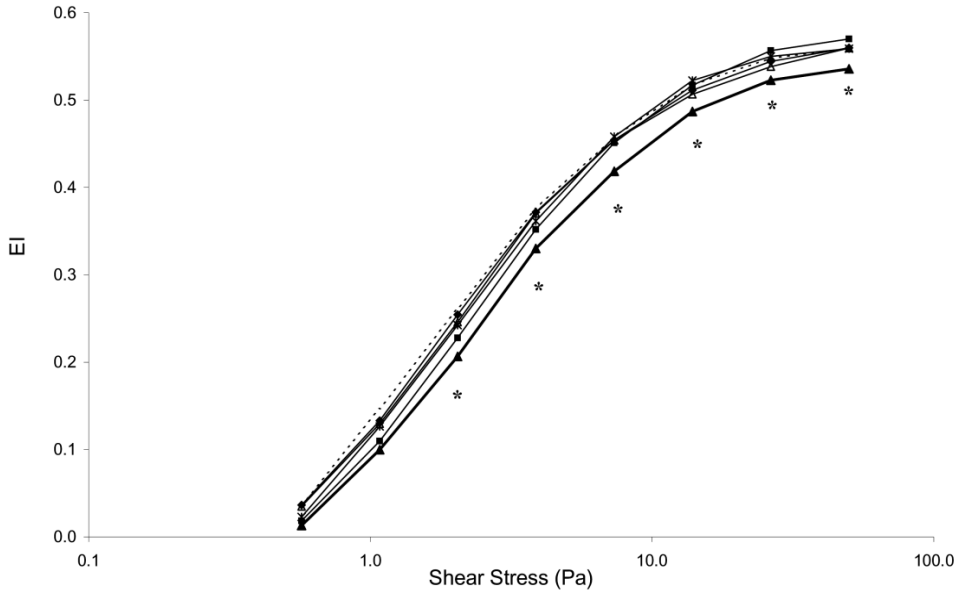

Figure 6.1. Shear stress EI curves for fresh, refrigerated stored and thawed deglycerolized RBCs. The shear stress value is plotted on logarithmic axis and data represent the mean ± SD of ten RBC units. The EI over a shear stress range of 2.0 Pa to 50 Pa of refrigerated stored and thawed deglycerolized RBCs was significantly elevated for all time points, compared to fresh RBCs (* $p < 0.05$). Fresh (▲); Day 3 liquid storage (■); Day 21 liquid storage (⋆); Day 35 liquid storage (--); Day 0 postthaw storage (Δ); Day 2 postthaw storage (♦).

Osmotic fragility

The osmotic fragility index of cryopreserved RBCs, as represented by the osmolarity at half-maximum hemolysis, was markedly enhanced ($p < 0.01$) in comparison to both fresh and refrigerated stored RBCs (Table 6.2). These results indicate that cryopreserved RBCs are more fragile than fresh and refrigerated stored RBCs. During storage, the osmotic fragility index of cryopreserved and refrigerated stored RBCs did not change significantly.

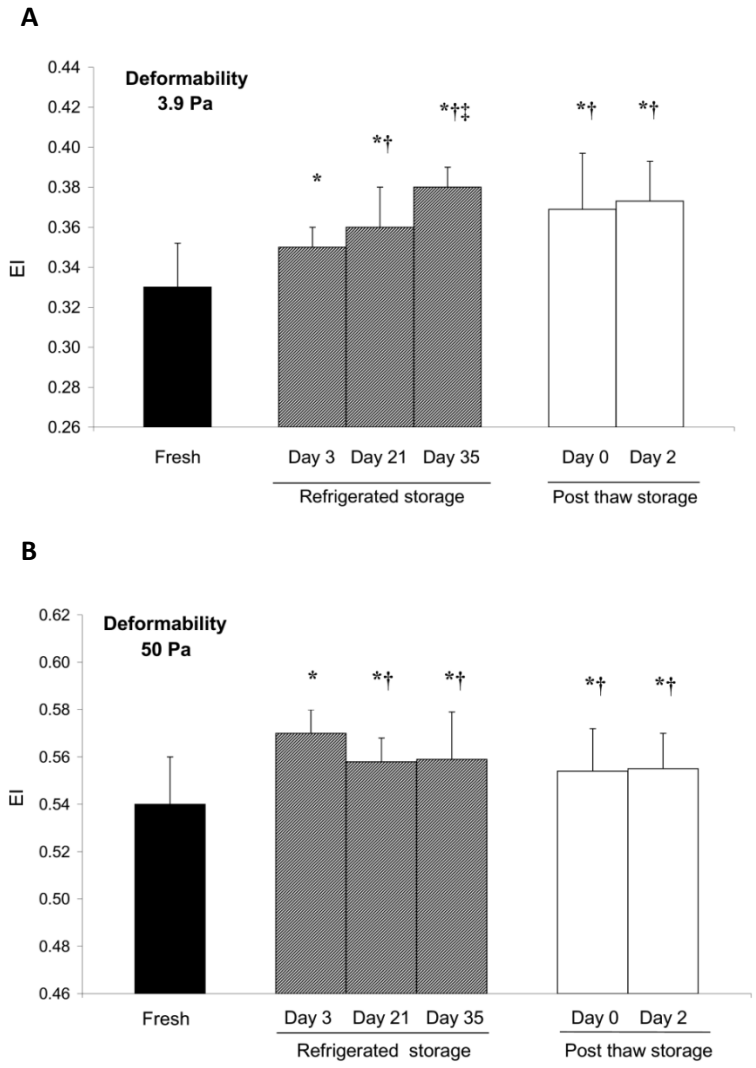

Figure 6.2. Deformability for two representative shear stress levels as a function of storage time. (A) EI at a shear stress of 3.9 Pa. (B) EI at a shear stress of 50 Pa. Values are expressed as the mean ± SD of ten RBC units. Significant changes are illustrated in the figure ($p < 0.05$); * significantly different from fresh RBCs, † significantly different from day 3 refrigerated stored RBCs, ‡ significantly different from day 21 refrigerated stored RBCs

Hematological variables

Cryopreserved RBCs had a considerably higher MCV ($p < 0.05$) and a lower MCHC ($p < 0.01$) compared to fresh and refrigerated stored RBCs (Table 6.2). During storage, the MCV of refrigerated stored RBCs was significantly enhanced. At the end of storage, the MCV and MCHC of cryopreserved RBCs differed from refrigerated stored RBCs by 15.7 % and respectively 20.8 %.

The hemolysis was generally higher in cryopreserved RBC units than in refrigerated stored RBC units (Table 6.2). Moreover, during storage the hemolysis increased with 1.7-fold in cryopreserved RBC units ($p < 0.05$) and with 2.2-fold in refrigerated stored RBC units ($p < 0.01$). As a result, after 2 days of post-thaw storage the hemolysis in the RBC unit slightly exceeded the allowable limits, whereas after 35 days of refrigerated storage the hemolysis in the RBC unit clearly remained below these limits (i.e., 0.8% in Europe and 1% in the United States).[3,4]

The ATP content of cryopreserved RBCs was similar to that of fresh and 3- or 21-day refrigerated stored RBCs (Table 6.2). During storage, the ATP content of cryopreserved and refrigerated stored RBCs reduced with 8.6 and 53.4% respectively. As a result, the ATP content at the end of refrigerated RBC storage was significantly different from that of fresh and cryopreserved RBCs. None of the aerobic and anaerobic blood cultures of cryopreserved RBC units showed evidence of bacterial contamination.

6.4. Discussion

The RBC aggregability and deformability are important hemodynamic determinants. Alterations in RBC rheology, that is, enhanced RBC aggregability and reduced deformability, have been observed in a variety of pathological states such as sepsis, myocardial ischemia, renal failure, inflammation, diabetes mellitus, obesity, hypertension, sickle cell disease and malaria.[15,19,20,29,30] Throughout the years, the rheologic properties of refrigerated stored RBCs have been extensively investigated.[31-40] So far, the rheologic properties of cryopreserved RBCs have scarcely been investigated.[41-43] The current study was undertaken to explore the rheologic features and hematologic variables of HGM cryopreserved RBCs and to compare this to conventional refrigerated stored and fresh RBCs.

In this study the aggregability of cryopreserved RBCs was markedly reduced, in comparison to that of refrigerated stored and fresh RBCs. The time necessary to induce aggregation was also slightly prolonged after deglycerolization, compared to refrigerated stored RBCs. In circulating blood, this may result in less effective transport of RBCs in the microcirculation. The ability of RBCs to aggregate is dependent on cellular properties and the composition of the suspension medium.[20] In our study the suspension medium was standardized for all samples, indicating that the observed differences were caused by alterations in cellular properties only. Cell morphology is a major determinant of RBC aggregability. Cell swelling suppresses cell contact and subsequently Rouleaux formation.[44,45] Previously, it has been shown that the freeze-thaw (wash) process made the RBC membrane permeable to cations and hence induced cell swelling.[9,46] Our study supports these results, as the MCV of cryopreserved RBCs markedly exceeded the MCV of fresh and refrigerated stored RBCs. This gain in RBC volume, as obtained by the freeze-thaw-wash process, could be responsible for the observed reduction in aggregability. Although the clinical relevance of altered RBC aggregability is an ongoing debate,[47] RBC swelling during storage is probably irrelevant, as long as it is reversible upon transfusion. In vitro data obtained from refrigerated stored RBCs support the hypothesis that RBC swelling can be reversed in plasma.[40]

The RBC ability to deform depends on the cytoplasmic viscosity of the cell, which is reflected by the MCHC, as well as on the overall cell shape and the viscoelastic properties of the cytoskeleton.[16] Our data showed no significant difference in the deformability between cryopreserved and long-term refrigerated stored RBCs. The deformation curves of both cryopreserved and refrigerated stored RBCs were, however, significantly elevated as compared to fresh RBCs. At a shear stress of 3.9 Pa an increasing deformability of refrigerated stored RBC and an increased deformability of cryopreserved RBC, with respect to fresh RBCs, was observed during storage, despite an enhanced MCV. At a shear stress of 50 Pa the deformability during refrigerated storage reduced, but was still elevated, whereas the deformability of cryopreserved RBCs was elevated and comparable to long-term refrigerated stored RBCs. These observations indicated that the RBC deformability in the low shear stress regions was less affected by changes in MCV. The deformability at a shear stress of 3.9 Pa, a shear stress which is predominantly found in the microcirculation,[48] is thus a direct reflection of the rigidity of the cell membrane. Altogether, the increased MCV and the resultant lowered MCHC value of cryopreserved RBCs did not seem to adversely

influence the RBC flexibility, because the deformability of cryopreserved RBCs was still higher than the deformability of fresh RBCs. The improved flexibility of 21- and 35-day refrigerated stored as well as that of cryopreserved RBCs, could be explained by di(2-ethylhexyl)phthalate (DEHP) leaking from the PVC storage bag. This plasticizer, which is added to the PVC to impart flexibility, is known to improve RBC storage by suppressing hemolysis, microvesiculation and morphology changes.[49-53] It was also shown that the presence of DEHP improved the flexibility of RBCs during long-term refrigerated storage.[54] We hypothesize that DEHP enhances the viscoelastic properties of the cytoskeleton, explaining the improved deformability of long-term refrigerated stored and cryopreserved RBCs, despite the gain in cell volume. The observation that the deformability at a shear stress of 3.9 Pa of both fresh and 3-day refrigerated stored RBCs was significantly lower than long-term refrigerated stored and cryopreserved RBCs further substantiates this hypothesis.

In the past, the RBC flexibility has been investigated after subjection of RBCs to subzero temperatures.[41-43] Nevertheless, in those studies the RBC storage conditions were not representative for current clinical standards, explaining the discrepancy between our results.

The osmotic fragility was significantly increased in cryopreserved RBCs compared to fresh and refrigerated stored RBCs. This indicates that the freeze-thaw-wash process made the RBCs more fragile. Moreover, the hemolysis was also significantly higher during post-thaw storage. At the end of storage, the hemolysis in cryopreserved RBC units exceeded the allowable limits. Previous studies demonstrated, however, that hemolysis during 48 hours of post-thaw storage remained clearly within the allowable limits.[5,9,23] In our study, polypropylene containers were used for storage of thawed RBCs. It cannot be ruled out that the lower gas permeability or the absence of DEHP plasticizer of these containers, was responsible for the increased hemolysis observed in our experiments.[55]

ATP as an energy source is important for the overall functioning of the RBC. Loss of ATP is associated with more rigid cell membranes, echinocyte shape change, enhanced cation permeability, loss of vasodilatation control, exposure of phosphatidylserine on the external RBC membrane, microvesiculation, and decreased RBC viability.[56-64] In the past it was shown that the RBC ATP content must be at least 2.7 μmol per gram Hb to have a 90 percent chance of acceptable in vivo survival (24-hr in vivo recovery of 75% or higher).[65,66] In our study, the ATP content of 35-day refrigerated stored RBCs was slightly below this limit, whereas the ATP content of cryopreserved RBCs remained close to those of fresh

RBCs. These results are in line with current findings, which showed a minimal loss of ATP in cryopreserved RBCs.[9,12,23] More recently, it was indicated that the RBC ATP content plays a prominent role in restoring oxygen deficits in the microcirculation.[67,68] Transfusion of cryopreserved RBCs with physiological ATP content would therefore favor the RBC viability and the oxygen delivery to the tissues.

In transfusion medicine, cryopreserved RBCs are a valuable blood resource for controlling an inventory in situations where the RBC availability is limited or unpredictable. This is the case for storage of RBC with rare blood types or for usage in military settings and occasionally during civil disasters.[5,69,70] Routine usage of cryopreserved RBCs in transfusion medicine is limited due to the more expensive, time consuming and less efficient nature of this preservation method. Consequently, the unfamiliarity with regard to the quality of cryopreserved RBCs has further limited clinical usage over the years. Yet, cryopreserved RBCs showed satisfactory in vitro quality and posttransfusion in vivo survival,[1,9,12,71] Improving the RBC freezing technology with preservation of the current quality, could ultimately make cryopreserved RBCs more utilizable for modern transfusion medicine.

Our data demonstrate that although cryopreserved RBCs are more fragile than refrigerated stored and fresh RBCs, the freeze-thaw-wash process did not adversely affect the ATP content or the aggregability and deformability of cryopreserved RBCs. From a rheologic point of view, we concluded that cryopreserved RBCs are a valuable alternative to refrigerated stored RBCs for usage in transfusion medicine.

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